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ROBERT FEULGEN LECTURE

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Cell cycle maintenance and biogenesis of the Golgi complex

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Abstract How organelle identity is established and maintained, and how organelles divide and partition between daughter cells, are central questions of organelle biology. For the membrane-bound organelles of the secretory and endocytic pathways [including the endoplasmic reticulum (ER), Golgi complex, lysosomes, and endosomes], answering these questions has proved difficult because these organelles undergo continuous exchange of material. As a result, many "resident" proteins are not localized to a single site, organelle boundaries overlap, and when interorganellar membrane flow is interrupted, organelle structure is altered. The existence and identity of these organelles, therefore, appears to be a product of the dynamic processes of membrane trafficking and sorting. This is particularly true for the Golgi complex, which resides and functions at the crossroads of the secretory pathway. The Golgi receives newly synthesized proteins from the ER, covalently modifies them, and then distributes them to various final destinations within the cell. In addition, the Golgi recycles selected components back to the ER. These activities result from the Golgi's distinctive membranes, which are organized as polarized stacks (*cis* to *trans*) of flattened cisternae surrounded by tubules and vesicles. Golgi membranes are highly dynamic despite their characteristic organization and morphology, undergoing rapid disassembly and reassembly during mitosis and in response to perturbations in membrane trafficking pathways. How Golgi membranes fragment and disperse under these conditions is only beginning to be clarified, but is central to understanding the mechanism(s) underlying Golgi identity and biogenesis. Recent work, discussed in this review, suggests that membrane recycling pathways operating between the Golgi and ER play an indispensable role in Golgi maintenance and biogenesis, with the Golgi dispersing and reforming through the intermediary of the ER both in mitosis and in interphase when membrane cycling pathways are disrupted.

Key words Golgi complex · Mitosis · Golgi/ER recycling

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Golgi enzyme enrichment and retention within Golgi stacks

Newly synthesized protein and lipid destined for the Golgi complex or beyond originate in the endoplasmic reticulum (ER), where they are selectively packaged into pre-Golgi transport intermediates that bud off from the ER and target to the Golgi complex (Fig. 1). Because pre-Golgi transport intermediates undergo maturation (by recycling of selected components back to the ER; Tang et al. 1995; Klumperman et al. 1998; Martínez-Menárguez et al. 1999), are capable of homotypic fusion (Presley et al. 1997), and track into the Golgi (Presley et al. 1997; Scales et al. 1997), they are generally thought to be the direct precursors of Golgi elements, with Golgi cisternae formed by continuous maturation/differentiation of pre-Golgi intermediates. The Golgi complex, in this view, represents a dynamic steady-state system of ER-derived membranes undergoing maturation, recycling, and transport (Mironov et al. 1997; Glick and Malhotra 1998; Lippincott-Schwartz et al. 1998). These processes are thought to give rise to the polarized entry (*cis*) and exit (*trans*) faces of the Golgi complex and to result in the selective enrichment of a variety of different proteins and lipids within Golgi membranes.

Among the proteins that are selectively enriched in Golgi membranes are glycosylation enzymes that orchestrate carbohydrate addition and remodeling of protein and lipid moving through the secretory pathway. An estimated 100–200 different glycosyltransferase enzymes

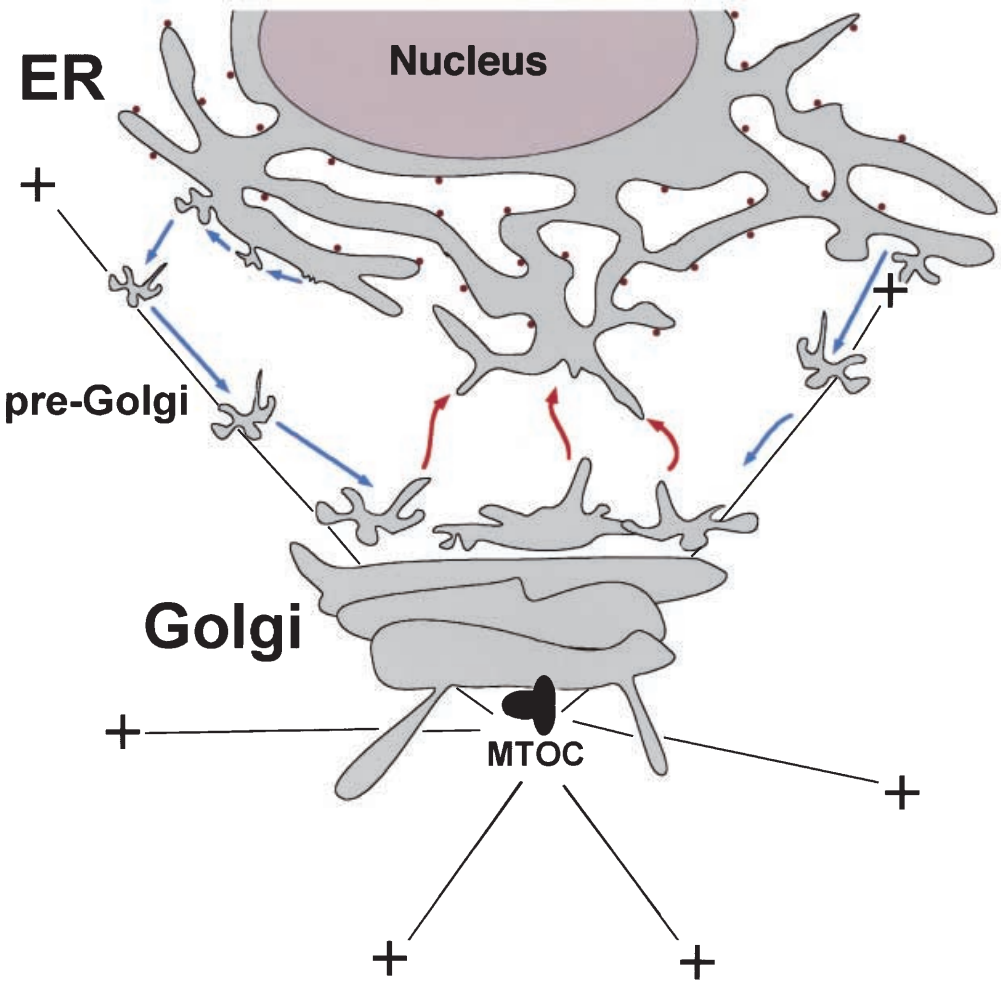

Fig. 1 Diagram of the endoplasmic reticulum (*ER*)/Golgi system. Newly synthesized proteins are synthesized in the ER and are packaged into transport intermediates that bud off from multiple sites on the ER. The pre-Golgi intermediates (*pre-Golgi*) track into the Golgi region along microtubules, which extend plus end-directed out from the microtubule organizing center (*MTOC*). Pre-Golgi intermediates deliver their cargo to the Golgi complex (*Golgi*). Forward trafficking of membrane is balanced by a retrograde pathway that brings selected membrane components back to the ER

are distributed throughout the Golgi stack and are involved in the ordered remodeling of *N*-linked oligosaccharide side chains and the addition of *O*-linked glycans and oligosaccharide portions of proteoglycans and glycolipids. Collectively, they represent the major proportion of resident Golgi membrane proteins and are significantly concentrated in Golgi compared to other membranes. Quantitation of one such enzyme, galactosyltransferase, tagged with green fluorescent protein (GFP) in living cells using fluorescent imaging techniques revealed a 15-fold concentration of this protein in Golgi relative to ER membranes with no significant pool on the plasma membrane (Zaal et al. 1999).

The major contribution of glycosylation enzymes to Golgi structure and function has led to a widespread interest in understanding how these enzymes are retained in the Golgi in the face of continuous flow of protein and lipid into and out of this organelle. Three possibilities have been proposed. Golgi enzymes could: (1) be tethered and immobilized within Golgi cisternae through interaction with a Golgi matrix, (2) form oligomers that are too large to enter forward or retrograde transport intermediates, or (3) be freely mobile and transported within constitutive Golgi cycling pathways. Research over the past few years favors the third of these models. Such studies have shown that Golgi enzymes are highly mobile in Golgi cisternae (Cole et al. 1996a), can rapidly diffuse between Golgi stacks via tubule interconnections (Sciaky et al. 1997; Polishchuk et al. 1999), and are capable of cycling *trans* to *cis* within the Golgi stack (Harris and Waters 1996; Love et al. 1998).

Golgi enzyme recycling within the Golgi complex

The mechanism whereby Golgi enzymes recycle within Golgi stacks is still unclear. Many proteins that are known to undergo similar cycling contain dibasic signals within their cytoplasmic domains (for example KKXX; Nilsson et al. 1989) that are thought to be recognized by a sorting machinery (including COPI; Letourneur et al. 1994) that mediates their entry into retrograde vesicles. Golgi enzymes, however, generally lack such retrieval signals within their cytoplasmic tails. These enzymes typically have a single, short hydrophobic membrane-spanning domain (approximately 14–16 amino acids), a short amino terminal cytoplasmic tail (less than 10 amino acids), and a large, carboxy terminal catalytic domain in the lumen of the Golgi (Gleeson 1998). A clue to the basis for their retention in the Golgi complex came with the observation that lengthening their transmembrane region (to 23 amino acids) caused them to no longer be retained in the Golgi complex

and to be delivered to the plasma membrane (Munro 1995). This finding raised the possibility that membrane partitioning of Golgi enzymes into different lipid environments within the Golgi could underlie recycling and retention of these proteins in Golgi membranes (Bretscher and Munro 1993; Simons and Ikonen 1997).

The Golgi is known to be a major site of lipid transition within cells. Its lipid composition is intermediate between that of the ER (whose lipids are enriched in glycerophospholipids) and the plasma membrane (whose lipids are enriched in cholesterol and glycosphingolipids; Fang et al. 1998). Various studies have indicated that the concentrations of sphingomyelin and cholesterol (which are 'thick' lipids) increases in a *cis*- to *trans*-Golgi direction, due in part to the Golgi's ability to drive glycosphingolipid synthesis. Therefore, Golgi enzymes could partition into thinner regions of the membrane bilayer due to the thinness of their transmembrane domains and be excluded from thicker regions that are enriched in sphingolipid and sterols destined for the plasma membrane. The finding that shortening the transmembrane domain of the plasma membrane targeted protein, VSVG, leads to its localization within the Golgi (Cole et al. 1998) is consistent with this possibility and suggests that the overall extent of membrane recycling in the Golgi is a constitutive process that operates independently of specific retrieval determinants.

While most models for Golgi protein recycling invoke the activity of retrograde vesicles (Pelham 1995; Rothman and Wieland 1996; Glick et al. 1997), an alternative possibility is that recycling occurs by diffusion of Golgi enzymes through the extensive tubule connections between Golgi stacks (Mironov et al. 1997; Lippincott-Schwartz et al. 1998). In this view, the processes of sequential modification of forward-moving cargo by Golgi enzymes coupled with changes in lipid composition across the Golgi stack would drive the spatial organization of otherwise highly mobile Golgi enzymes within Golgi membranes. Further work characterizing the mechanism(s) by which proteins are transported and sorted within the Golgi complex are needed in order to distinguish between this and other models for Golgi recycling.

What functions are served by Golgi enzyme recycling within Golgi stacks? Without such a process, Golgi enzymes undoubtedly would be swept out of the Golgi complex along with forward-moving secretory cargo since they are not tethered or immobilized within Golgi cisternae. Recycling of Golgi enzymes also is likely to be crucial for the glycosylation activities of the Golgi complex. As cargo passes through the Golgi stack, continuous retrieval of Golgi processing enzymes to sites where substrate is first available for modification is likely to enhance the efficiency of glycosylation and to ensure that enzymes are available to act sequentially in modifying their precursors.

Golgi enzyme recycling to the ER

A retrograde pathway from the Golgi back to the ER (Pelham 1995) maintains the surface area of the ER in the face of continuous membrane outflow into the secretory pathway. In addition, it is thought to serve as a retrieval pathway to prevent loss of ER resident proteins into the secretory pathway, as reported for soluble ER proteins containing the C-terminal tetrapeptide sequence KDEL (or HDEL; Lewis and Pelham 1992), which are recycled back to the ER after binding to KDEL receptor in the Golgi (Miesenböck and Rothman 1995). It also allows ER export machinery to be reutilized as shown by the selective recycling of cargo receptors (including ERGIC53 and the p24 family of proteins; Tang et al. 1995; Dominguez et al. 1998; Nickel et al. 1998; Hauri et al. 2000) and of t-SNAREs involved in forward trafficking through the secretory pathway (Lewis and Pelham 1996).

Given that Golgi enzymes are capable of recycling within Golgi membranes, do these enzymes ever cycle back to the ER? The first evidence that this was the case came with observations of the effects of the drug brefeldin A (BFA; Klausner et al. 1992) on cells. BFA inhibits the activation of ARF1 (Donaldson et al. 1992b; Helms and Rothman 1992), a raslike GTPase whose GTP-bound form is required to initiate COPI binding to membrane (Donaldson et al. 1992a). Membrane association of these cytosolic coat proteins regulates sorting and recycling within pre-Golgi and Golgi intermediates (Rothman and Wieland 1996; Schekman and Orci 1996; Lippincott-Schwartz et al. 1998). In the absence of such binding in BFA-treated cells, membrane trafficking is deregulated, with anterograde, ER-to-Golgi traffic inhibited and retrograde, Golgi-to-ER traffic accelerated (Klausner et al. 1992). Golgi enzymes redistribute into the ER during BFA treatment indicating that Golgi proteins are capable of entering a retrograde pathway back to the ER.

To determine whether Golgi proteins normally recycle back to the ER in the absence of membrane trafficking perturbants, Cole et al. (1998) developed a retrograde transport assay based on the capacity of the ER to retain misfolded proteins. The ER is uniquely enriched in chaperones and folding enzymes that facilitate folding and unfolding reactions and ensure that only correctly folded and assembled proteins leave this compartment (Hammond and Helenius 1995). In this assay, the luminal domain of the temperature-sensitive viral glycoprotein VSVGts045 was fused to Golgi or plasma membrane targeting domains. Addition of the VSVGts045 ectodomain was shown to be sufficient to retain the fusion proteins in the ER at 40°C, where they are misfolded. At the permissive temperature of 32°C, the fusion proteins correctly localized to the Golgi complex or plasma membrane, indicating that the luminal epitope of VSVGts045 did not interfere with their proper targeting. When cells were shifted from 32°C back to 40°C, fusion proteins with the Golgi targeting domain, but not those destined for the plasma membrane, reaccumulated within the ER due to misfolding of the G protein ectodomain. Importantly, recycling was not induced by misfolding of the chimeras within the Golgi complex. Rather, the chimeras exhibited a misfolded phenotype only after their redistribution into the ER. These results suggested that

the chimeras normally cycle between the Golgi and ER, despite their Golgi localization phenotype, and while passing through the ER at 40°C become misfolded and retained.

More direct evidence that Golgi proteins undergo constitutive cycling through the ER was obtained in photobleaching experiments of cells expressing the Golgi enzyme galactosyltransferase tagged with GFP (GalTase-GFP). Using quantitative imaging techniques, Zaal et al. (1999) showed that GalTase-GFP resides in Golgi and ER membranes at steady-state. When the Golgi or ER pools of GalTase-GFP were selectively photobleached in the absence of new protein synthesis, recovery of the bleached pool was observed to be rapid and occurred at the expense of fluorescence from the non-bleached pool. These results indicated that the Golgi and ER pools of GalTase-GFP are constantly exchanging. Kinetic modeling of the data revealed that GalTase-GFP cycles between the ER and Golgi every 85 min, spending approximately 57 min in the Golgi complex and 28 min in the ER each cycle.

Given that at least some Golgi-localized proteins recycle back to the ER as revealed in the above experimental systems, what functions might such cycling serve? The ER is unique in its content of folding enzymes and chaperones that catalyze dynamic folding/unfolding reactions, so recycling to the ER could be important for monitoring the fidelity of proteins that spend most of their lifetime in distal secretory compartments. For Golgi enzymes, in particular, it could allow them to be periodically exposed to the folding machinery in the ER where they could undergo further modification/repair and/or degradation (Cole et al. 1998).

An additional role of Golgi protein recycling to the ER is its potential influence on the distribution and organization of the Golgi complex itself. If the bulk of Golgi membrane components constitutively recycle to the ER, then the integrity of the Golgi complex will be critically dependent upon the return of these components from the ER. A wide variety of conditions interfere with delivery of secretory cargo from the ER into the Golgi complex, including treatments with BFA, nocodazole, okadaic acid, and overexpression of mutant forms of Sar1p and ARF1 (which are small GTPases that recruit the cytosolic coat complexes of COPII and COPI onto membranes, respectively). These also result in dispersal of Golgi membranes (Lippincott-Schwartz et al. 1990; Lucocq et al. 1991; Dascher and Balch 1994; Teal et al. 1994; Peters et al. 1995; Cole et al. 1996b; Storrie et al. 1998), which is consistent with the idea that the bulk of Golgi membrane components constitutively cycle through the ER.

Golgi remodeling/dispersal in response to perturbants of ER/Golgi membrane cycling

A well-studied example of a condition that interferes with ER-to-Golgi transport and disrupts Golgi organization is microtubule disruption by nocodazole (Cole et al. 1996b; Storrie et al. 1998). This treatment blocks the translocation of ER-derived pre-Golgi transport intermediates into the centrosomal region where the Golgi complex normally resides (Presley et al. 1997). Golgi enzymes in nocodazole-treated cells redistribute within 60–90 min to numerous peripheral sites where they form mini Golgi stacks. Time-lapse and quantitative imaging techniques have shown that under these conditions a discrete number of ministacks (approximately 200) are generated in the cell periphery and remain constant thereafter. The ministacks form without tracking out from the Golgi region and reside adjacent to ER exit sites. Because such ministacks also form during BFA-washout into nocodazole, they must be capable of forming de novo upon export of Golgi enzymes from the ER. These results suggest that Golgi ministacks in nocodazole-treated cells form through the intermediary of the ER via bulk recycling of Golgi enzymes and other components. Because microtubules are disrupted, recycling Golgi proteins fail to be translocated back into the centrosomal region upon export out of the ER and thus build Golgi ministacks at ER exit sites.

A different example where perturbation of ER-to-Golgi transport affects Golgi structure is the overexpression of mutant Sar1p. ER export is known to be inhibited without effects on retrograde traffic in cells expressing a predominantly GDP-bound mutant of Sar1p (Barlowe et al. 1994; Shima et al. 1998). Within a few hours of expression of this protein in cells, Golgi enzymes became trapped in the ER and Golgi structures disappear (Storrie et al. 1998; Zaal et al. 1999). ER resident glycoproteins become modified by Golgi enzymes under these conditions. Because the rate at which Golgi proteins appear in the ER is too fast to be explained by trapping of newly synthesized Golgi enzymes (whose half-lives are usually over 15 h), the data indicate that Golgi enzymes redistribute into and function within the ER when ER exit is blocked by mutant Sar1p expression.

The two perturbations described above, nocodazole treatment and mSar1p expression, disrupt ER/Golgi trafficking pathways at different steps. Whereas overexpression of mSar1p blocks ER export and retains recycled Golgi enzymes in the ER, nocodazole treatment allows Golgi enzymes that cycle through the ER to accumulate in pre-Golgi intermediates that are trapped in the cell periphery due to the absence of microtubules. Despite these differences, both perturbants cause Golgi dispersal/fragmentation by a common pathway involving the ER.

Golgi breakdown/reassembly during mitosis

In mammalian cells, the Golgi complex breaks down and then reassembles during mitosis. The traditional model for this process postulates a direct breakdown of Golgi membranes into smaller units by continued budding and inhibited fusion (Warren 1993). In this view,

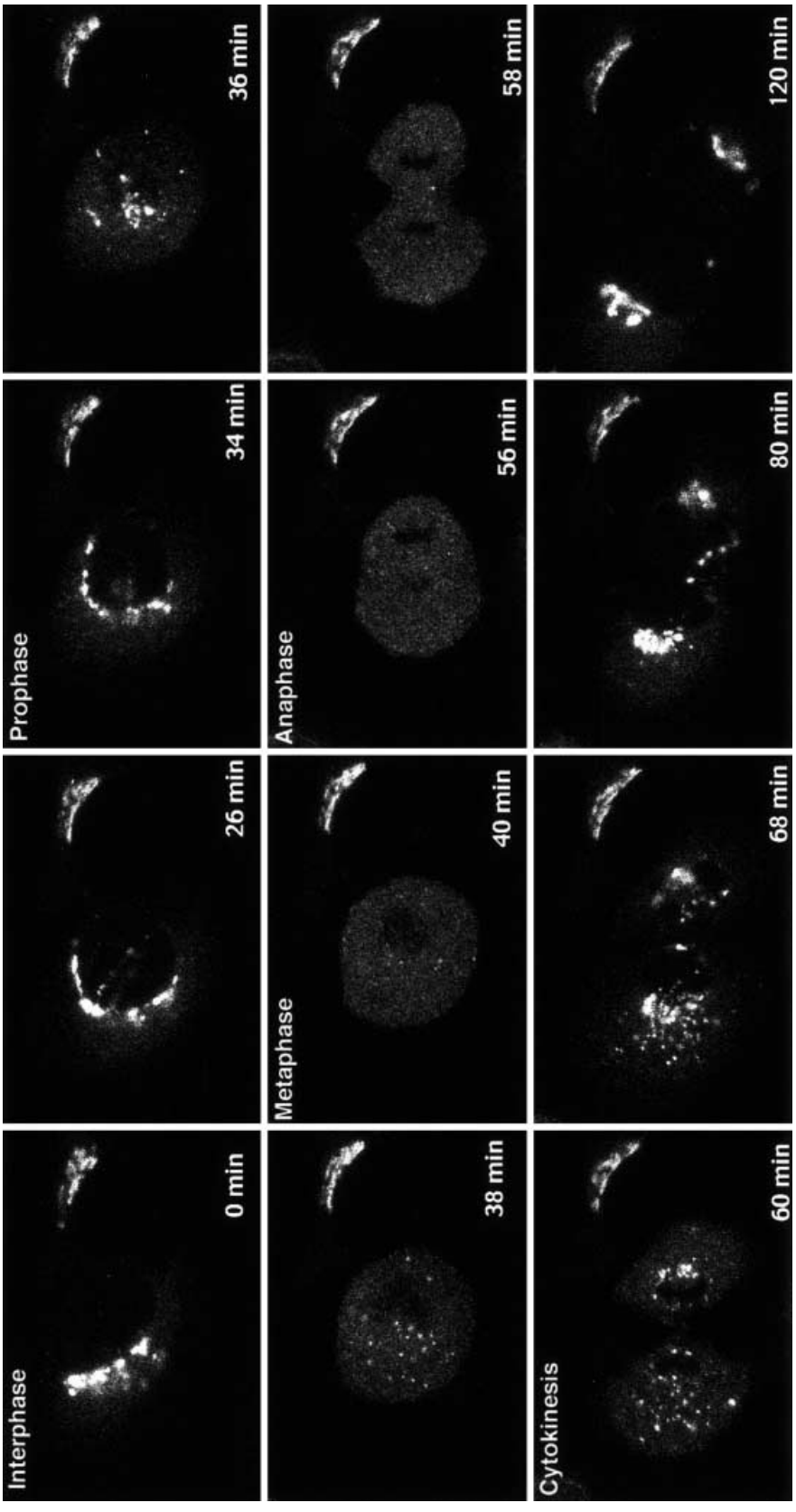

Fig. 2 Time-lapse sequence of HeLa cell expressing galactosyltransferase tagged with green fluorescent protein (GalTase-GFP) going through mitosis. Note that the Golgi disperses and reassembles during mitosis through a multistage process involving fragmentation, complete dispersal, and then coalescence of newly formed fragments (for more details see Zaal et al. 1999)

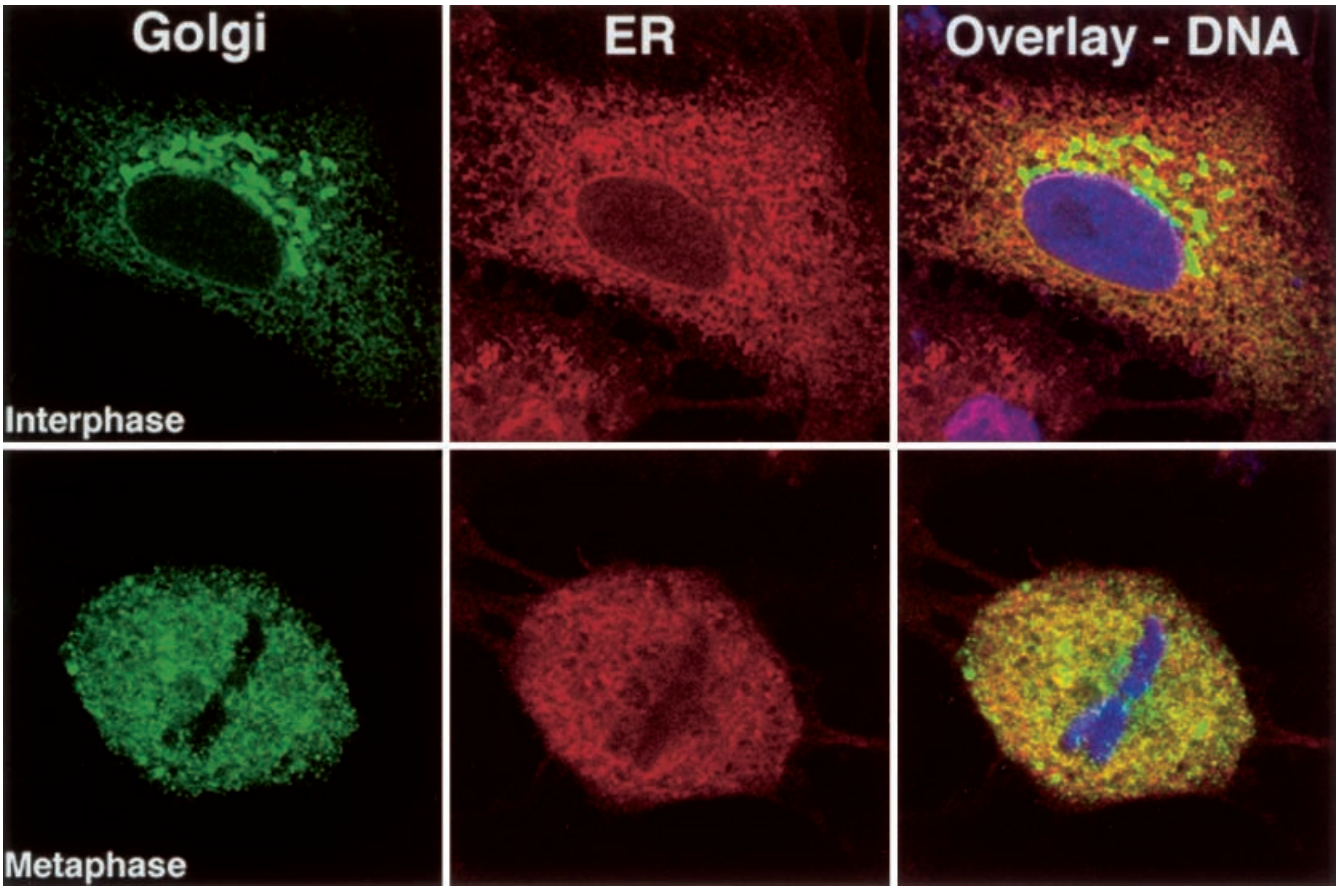

Fig. 3 Distribution of Golgi (GalTase-GFP, *green*) and ER (Louvard antibodies, *red*) proteins in fixed/permeabilized interphase and mitotic cells. *Yellow* shows overlap in the distribution of the two markers

Golgi-derived mitotic vesicles/clusters disperse throughout the cytoplasm, partition into daughter cells stochastically, and then fuse together at the end of mitosis. An alternative model is that Golgi breakdown and reassembly in mitosis results from alterations in Golgi protein cycling pathways through the ER. This is based on the fact that in mitosis forward trafficking out of the ER is blocked (Featherstone et al. 1985) and cytoplasmic microtubules are depolymerized (Wadsworth and Sloboda 1983), which are the same conditions that lead to Golgi disassembly/remodeling in interphase cells via changes in Golgi protein cycling pathways. Recent fluorescence imaging studies using GFP chimeras to visualize changes in Golgi organization during mitosis are consistent with the latter model and suggest that the Golgi is absorbed into and then re-emerges from the ER during mitosis (Zaal et al. 1999). Time-lapse imaging of the GFP-tagged Golgi proteins shows several discrete states of mitotic Golgi breakdown and reassembly (Fig. 2). These include the appearance of Golgi fragments at prophase when cytoplasmic microtubules begin to depolymerize, the complete loss of Golgi fragments and appearance of Golgi proteins in membranes widely dispersed throughout the cell during metaphase and telophase, and the reappearance of small Golgi fragments that subsequently coalesce into juxtanuclear Golgi elements during cytokinesis. Other time-lapse imaging studies of mitotic Golgi breakdown/reassembly using GFP chimeras have found similar stages of progression, although the extent to which Golgi proteins become widely dispersed within the cytoplasm during metaphase and telophase appears to be variable in different cell types (Shima et al. 1998; Terasaki 2000). The widely dispersed distribution of Golgi proteins in metaphase and telophase has been shown in immunofluorescence and electron microscopy studies to represent an ER localization (Thyberg and Moskalewski 1992; Zaal et al. 1999; see Fig. 3). Repetitive photobleaching experiments in living cells expressing Golgi-GFP chimeras further revealed that Golgi proteins in the ER of mitotic cells diffuse rapidly without residing in subdomains or separate non-continuous compartments (Zaal et al. 1999; see Fig. 4). Golgi structures did not reform during cytokinesis in cells expressing mutant Sar1p (Zaal et al. 1999), indicating that ER export was required for Golgi reassembly at the end of mitosis.

The finding that Golgi proteins in mitosis redistribute into the ER contrasts with earlier morphological and biochemical work suggesting that these proteins relocate to vesicles or membrane clusters distributed throughout the cytoplasm (Lucocq and Warren 1987; Jesch and Linstedt 1998). These disparate conclusions are understandable

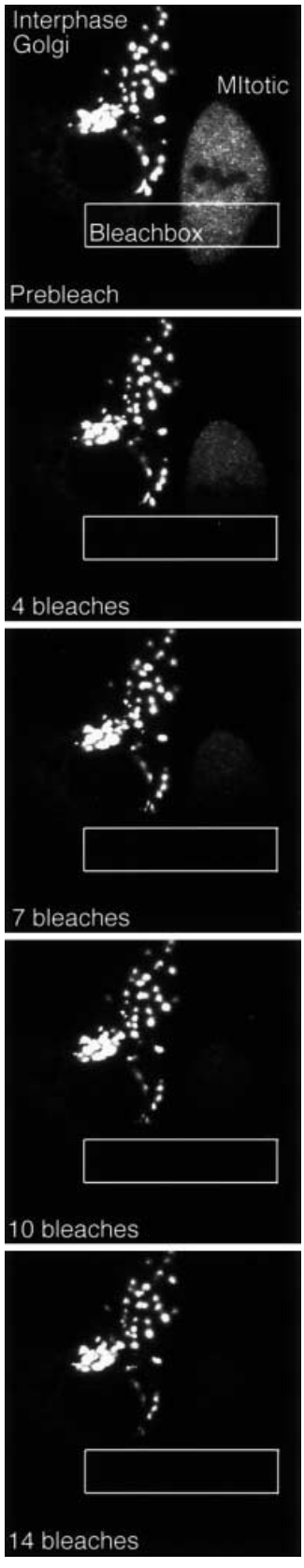

Fig. 4 Golgi proteins rapidly diffuse within mitotic membranes distributed throughout the cell. A defined area was repeatedly photobleached across GalTase-GFP-expressing cells. Note that after 14 cycles of repeated bleaching of the box, all fluorescence was lost from the mitotic cell, whereas fluorescence remained in the Golgi fragments of the interphase cell (see Zaal et al. 1999 for details)

given that mitotic Golgi disassembly is a sequential process involving the transformation of Golgi stacks into disorganized fragments/vesicles which then are absorbed into the ER (Nelson 2000). Early morphological studies used mitotic cells fixed at a single time point rather than following one cell through mitosis. Such studies, therefore, may have overlooked cells with Golgi proteins in the ER, since Golgi proteins are easier to identify when concentrated in fragments rather than dispersed. The finding in biochemical studies that different vesicle populations from mitotic cells are enriched in Golgi versus ER proteins (Jesch and Linstedt 1998) could result from the fact that thousands of cells at different stages of mitosis are analyzed from preparations of mitotic membrane homogenates, where membranes easily vesiculate and become fragmented in contrast to ER membranes in living cells.

Assuming Golgi proteins are absorbed into and reemerge from the ER during mitosis, what explains this phenomenon? An attractive idea discussed in more detail below is that it results from a reversible alteration of constitutive membrane cycling pathways connecting the ER and Golgi complex (Zaal et al. 1999). In this model, when ER export stops in mitosis (Featherstone et al. 1985) cycling Golgi proteins are trapped in the ER leading to loss of Golgi structure. Reformation of distinct Golgi elements within daughter cells at the end of mitosis would result from renewed exit of material out of the ER, with Golgi ministacks forming de novo and subsequently tracking into the centrosomal region upon microtubule repolymerization where they reform the juxtanuclear Golgi complex (see model in Fig. 5).

Mechanisms of Golgi fragmentation during mitosis

How ER export is blocked in mitosis is crucial for understanding the mechanism(s) underlying mitotic dispersal of Golgi membranes since once this occurs Golgi proteins constitutively cycling through the ER become trapped in this compartment. To address this question, Farmaki et al. (1999) studied the characteristics of the COPII coat subunit Sec13, whose assembly with other COPII subunits onto membranes at ER exit sites is widely recognized as crucial for ER export (Barlowe 1998). They found that in mitotic cells COPII failed to bind to ER exit sites. Whether inhibition of COPII activity in mitotic cells results from a direct inactivation of Sar1p, which recruits the COPII coat subunits Sec23/24 and Sec13/31 onto membranes (Schekman and Orci 1996), or to inhibition of Sec12-mediated nucleotide exchange of GTP onto Sar1p will be important to address in future studies.

Another regulatory point affected in mitotic cells is retrograde traffic from Golgi to ER. Quantitative imaging data of GalTase-GFP trafficking in mitotic cells revealed that the basal rate of retrograde traffic of GalTase-GFP (i.e., 1.8% per min) is accelerated at least

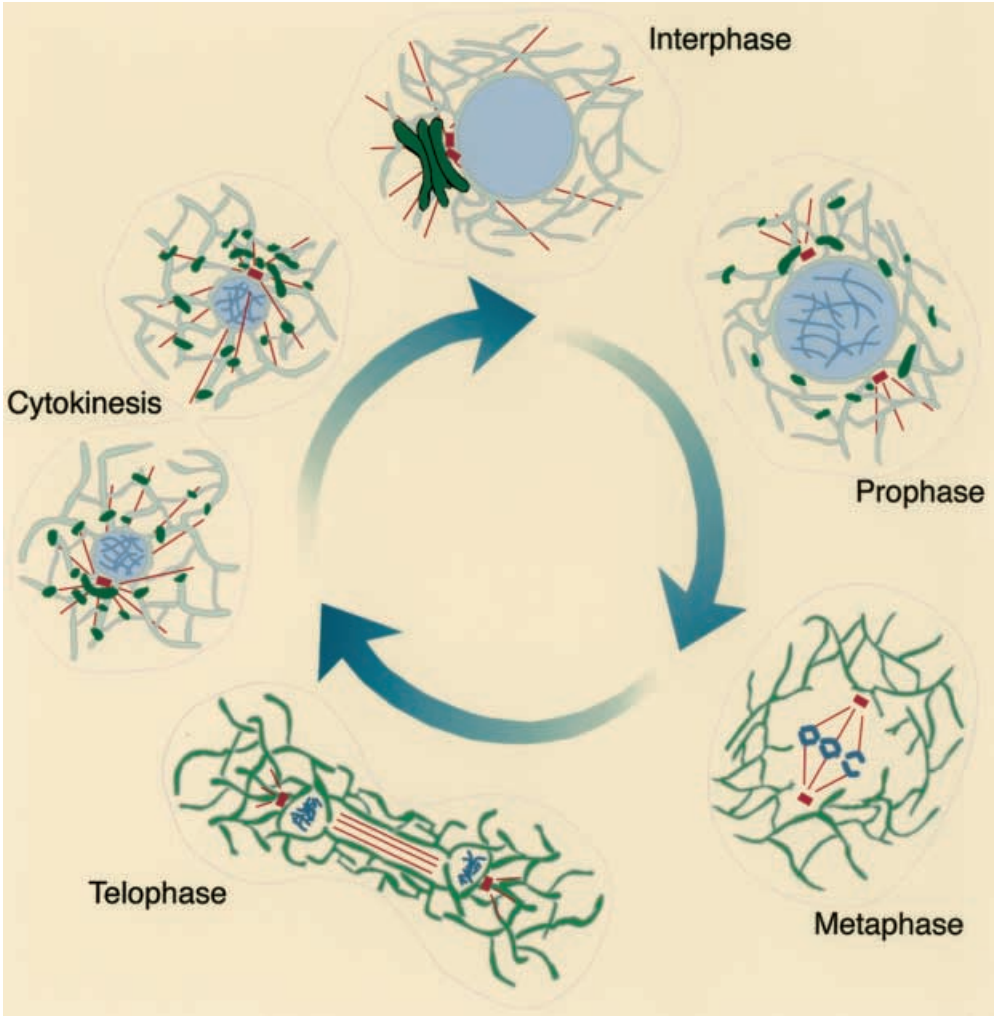

Fig. 5 Model for Golgi breakdown and reassembly. In interphase, Golgi proteins are concentrated in membranes of the Golgi complex (*dark green*) but are also present in the ER (*light green*), undergoing constitutive cycling between the two. During prophase, cytoplasmic microtubules depolymerize. In the absence of cytoplasmic microtubules, pre-Golgi structures (which return recycling Golgi proteins back to the Golgi complex) can no longer track back to the Golgi region near the MTOC. As a result, Golgi proteins accumulate in these structures, which form at numerous scattered sites in close association with the ER. During metaphase, ER export is inhibited and retrograde transport accelerated. This leads to the rapid absorption of Golgi proteins into the ER. Golgi proteins remain in the ER through telophase where they diffuse rapidly throughout the ER's interconnected membranes. At cytokinesis, export from the ER is no longer blocked. Highly mobile Golgi proteins exit the ER at widely dispersed ER exit sites, thereby ensuring equal partitioning of Golgi proteins within daughter cells. Golgi protein-enriched membranes at ER exit sites then use newly formed microtubule arrays organized around the MTOC to translocate inward to a central site in each daughter cell, thereby reforming centralized Golgi structures

20-fold in mitosis based on the rapid decline in Golgi fragment-associated fluorescence seen when Golgi proteins redistribute into the ER (Zaal et al. 1999). This suggests that some regulatory mechanism accelerates retrograde traffic during this period. Given that retrograde traffic is increased in BFA-treated cells, when nucleotide exchange onto ARF1 is inhibited (Donaldson et al. 1992b), it is worth examining whether the activation cycle of ARF1 is affected during mitosis at the time retrograde transport of Golgi proteins into the ER is accelerated.

Before Golgi proteins redistribute into the ER in mitotic cells, they disperse into numerous peripheral fragments distributed throughout the cytoplasm. Experimental systems dissecting the mechanisms of Golgi fragmentation have pointed to a role for Cdc2 kinase and mitogen-activated protein kinase kinase (MEK1) in this process (Acharya et al. 1998; Lowe et al. 1998). Evidence that MEK1 activity plays a role in initiating Golgi fragmentation has been suggested by the finding that a specific inhibitor of MEK1, PD 098059, blocks Golgi fragmentation in permeabilized cells incubated with mitotic cytosol (Acharya et al. 1998). Moreover, when MEK1 is phosphorylated during mitosis, it appears to promote MEK1 activity toward substrate(s) other than ERK1/2 that are involved in Golgi fragmentation (Colanzi et al. 2000). Whereas the MEK1 target(s) involved in mitotic Golgi fragmentation remain to be uncovered, the target of Cdc2 kinase has been well characterized. Cdc2 activi-

ty in mitosis has been shown to lead to phosphorylation of GM130, a protein involved in maintaining the structural organization of the Golgi complex (Lowe et al. 2000). Upon phosphorylation, GM130 is unable to interact with other Golgi structural components, including GRASP65, p115, and giantin (Linstedt and Hauri 1993; Levine et al. 1996; Barr et al. 1998). This leads to the generation of Golgi fragments scattered throughout the cell which are thought to result from loss of Golgi integrity.

How mitotic Golgi 'fragments' arise during mitosis is unclear. Large membrane aggregates of diameter greater than 0.5 μm are too large to freely diffuse through the cytoplasm (Luby-Phelps 1994) and cytoplasmic microtubules, which could mediate movement of these structures, are depolymerized (Wadsworth and Sloboda 1983). One possibility is that Golgi 'fragments' arising during mitosis are not the direct breakdown products of the Golgi complex per se, but represent pre-Golgi intermediates enriched in recycled Golgi proteins derived from the ER which are unable to mature and differentiate into Golgi elements when GM130 and other Golgi structural components are phosphorylated during mitosis. The fact that p115 and GM130 are found on pre-Golgi intermediates is consistent with this possibility (Nakamura et al. 1997). In this view, the underlying cause of Golgi fragmentation in mitosis mediated by Cdc2 kinase or MEK1 activity would be a disruption in both the forward pathway carrying membrane from peripheral ER exit sites into the centrosomal region of the cell and of the processes whereby these membranes fuse and differentiate into Golgi elements.

Conclusion

The view that organelles are pre-existent, forming solely by growth and division (Warren and Wickner 1996), has until recently been the paradigm for Golgi division during mitosis. Golgi partitioning into daughter cells under this reasoning, occurs by direct breakdown of Golgi membranes into vesicles and fragments, which then are inherited as discrete units in the cytoplasm. Recent studies, discussed in this review, question this model by showing that during mitosis Golgi proteins predominantly redistribute into the interconnected membrane system of the ER, where accurate partitioning of Golgi proteins is ensured. At the end of mitosis, Golgi proteins and cargo are exported out of the ER, reforming Golgi stacks de novo.

The dynamic redistribution of Golgi proteins into the ER during mitosis has been proposed to arise from constitutive recycling of Golgi proteins to the ER. Golgi proteins in interphase cells continuously transit between Golgi and ER membranes, so much so that perturbations that disrupt such cycling quickly lead to redistribution of Golgi proteins to the site of inhibition. Since protein export from the ER is blocked at mitosis, loss of Golgi structure over time in mitotic cells is likely to be due to entrapment of constitutively cycling Golgi proteins in the ER.

This view of the Golgi complex and its fate during mitosis assumes a fundamental relationship between Golgi-ER recycling pathways and Golgi biogenesis and maintenance, with the Golgi existing as a steady-state system exhibiting substantial recycling of Golgi proteins through the ER. Future work aimed at understanding this system will need to address the mechanism(s) underlying Golgi-ER recycling and its perturbation during mitosis. Specific areas to investigate include the roles of lipid partitioning, structural proteins that associate with Golgi membranes (including coat complexes and Golgi matrix components), retrieval signals, and other biochemical requirements that regulate entry into the Golgi-ER cycling pathway.

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